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Life history observations of the Illinois state endangered Enigmatic Cavesnail, *Fontigens antroecetes* (Hubricht, 1940) made under simulated cave conditions

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Abstract

The Enigmatic Cavesnail, *Fontigens antroecetes* (Hubricht, 1940), is a cave adapted hydrobioid snail listed as state endangered in Illinois. It is known from only one cave in Illinois, Stemler Cave, and from several caves in the eastern Ozark ecoregion of Missouri. Little is known about the snail's reproductive habits, embryological development, or growth rates. I attempted to gain basic life history information by breeding Enigmatic Cavesnails under simulated cave conditions in the laboratory. Six adult snails were collected from Stemler Cave and held in aerated containers of cave water with one or two cobbles from the cave stream. Containers of snails were housed in incubators set at the average cave water temperature of 13 °C. The snails produced 49 embryos in captivity over the course of 34 weeks. Eggs were deposited singly, attached to the underside of rocks within small pits or crevices. Nearly 82% of embryos developed to hatching. Mean estimated development time of embryos was 70.7 days. Survival of hatchling snails was poor. Limited data available from surviving hatchling snails suggests slow growth rates. The process was replicated with nine Enigmatic Cavesnails collected from Cliff Cave in St. Louis County MO. Captive Cliff Cave snails produced 34 embryos over 46 weeks and varied from the Stemler population in their oviposition behavior, with a majority of eggs deposited on the top surface of rocks. Cliff Cave snail embryos also had longer mean estimated development times (82.17 days).

Keywords

Captive breeding, Fontigentidae, gastropod, stygobiont

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Introduction

Subterranean ecosystems are likely the most widespread nonmarine environments on Earth, yet specialized subterranean organisms are among the least documented and studied groups (Mammola et al. 2019). Within stygobitic communities (i.e., restricted to aquatic subterranean habitats), snails are one of the least studied taxa and face elevated risk of extinction due to their limited geographic distribution and habitat degradation, including nutrient enrichment and groundwater contamination (Gladstone et al. 2021). The members of the gastropod clade Hydrobioidea, which includes several families that are collectively referred to as hydrobioids, are extensively adapted to subterranean aquatic habitats (Hershler and Holsinger 1990). These habitats include cave streams, small shallow aquifers that discharge as seep springs (hypotelminorheic habitats), interstitial spaces beneath stream beds (hyporheic zones), and deep aquifers (phreatic zones) (Culver and Pipan 2009; Culver et al. 2012; Alvear et al. 2020). Many hydrobioid species are restricted to springs (crenobionts), living at the interface between subterranean and surface ecosystems (Marenti and Pezzoli 2019). A few species of the North American hydrobioid genus *Fontigens* are found in both cave streams and springs (Steward and Dillon 2004), with surface populations exhibiting full pigmentation and cave-dwelling population showing some features typical of stygobionts, such as reduced pigmentation (Hershler et al. 1990). In one case, a styogobitic hydrobioid thought to be endemic to a single cave was found to inhabit two nearby springs (Delicado 2018). Basic information about life history and the ecological role of most stygosnails is lacking. It is generally assumed that subterranean gastropods feed on detritus (Culver 2005). Simons et al. (2003) used C and N isotope abundance to show that the hydrobioid *Fontigens tartarea* Hubricht, 1963 likely feeds on epilithic microbial biofilms in caves.

The Enigmatic Cavesnail, *Fontigens antroecetes* (Hubricht, 1940) is a minute stygobitic hydrobioid limited to caves in the eastern edge of the Ozarks karst region, USA (Fig. 1). In Missouri, the Enigmatic Cavesnail has been reported from several caves in Perry County, and a single site in St. Louis County, Cliff Cave (Hershler et al. 1990; Wu et al. 1997). The only population known east of the Mississippi River is the type-locality, Stemler Cave in St. Clair County, Illinois (Lewis et al. 2003). *Fontigens antroecetes* has a NatureServe ranking of G2 (Imperiled), is listed as Endangered in Illinois (S1 ranking), and is a species of Conservation Concern in Missouri (S2 ranking). Baseline information on population density and habitat use in Stemler Cave are available (Taylor et al. 2013), however little else is known about the life history of *F. antroecetes*.

High levels of fecal coliforms and other contaminants have been documented in Stemler Cave (Taylor et al. 2000; Dodgen et al. 2017) and the resulting hypoxia has likely led to the extirpation of another stygobiont, the federally endangered Illinois Cave Amphipod (*Gammarus acherondytes*, Hubricht and Mackin 1940) from that cave system (Panno et al. 2006). In a recent review of actions and policies aimed at conservation of subterranean gastropod diversity in North America, Gladstone et al. (2021)

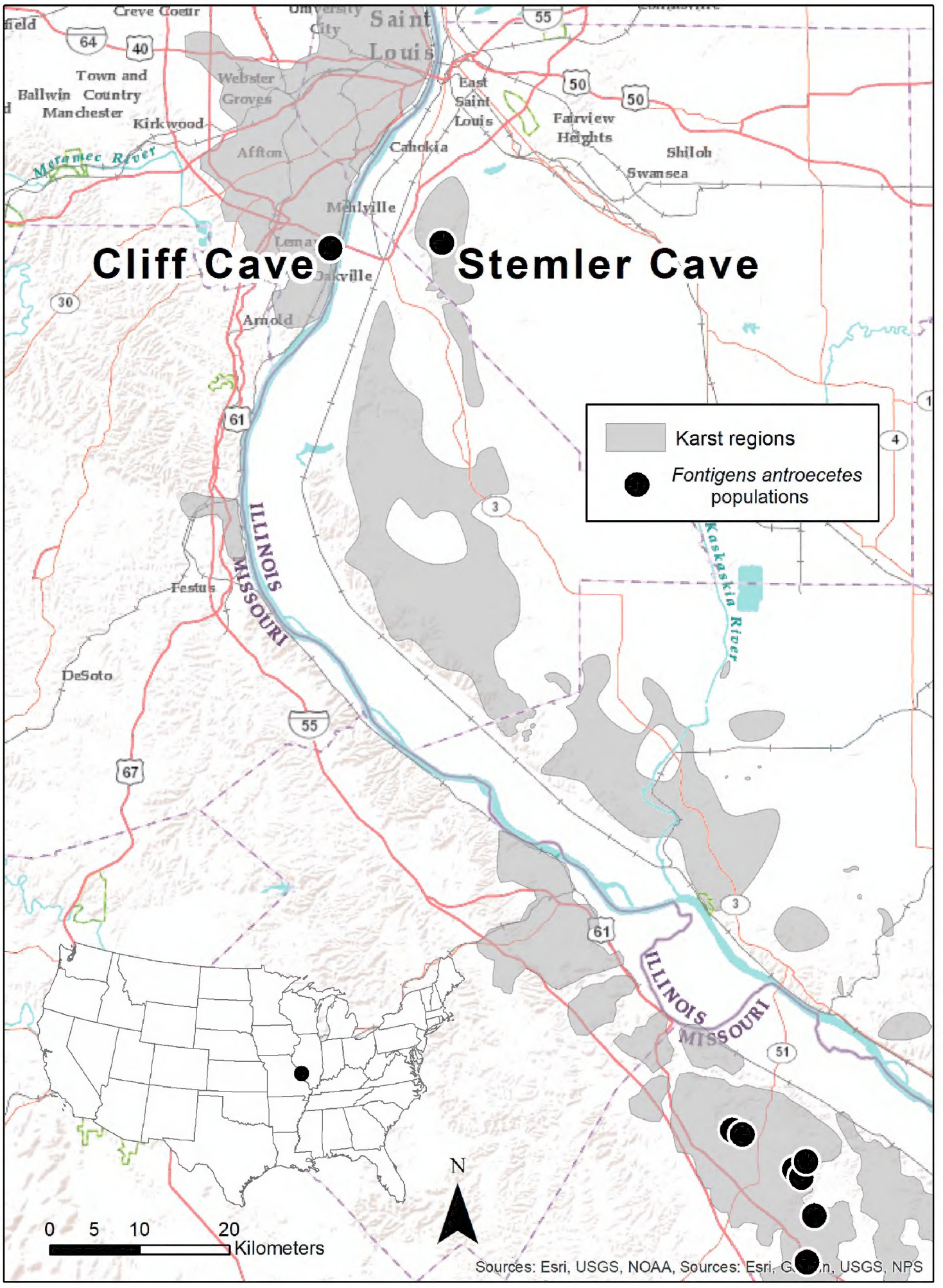

Figure 1. Distribution of the Enigmatic Cavesnail (*Fontigens antroecetes*). Gray areas represent karst regions. Black dots represent known populations.

highlighted the potential usefulness of captively propagating sygobitic snails in gaining information on life history and reproductive biology. Captive propagation could also be an important strategy in reintroduction efforts should isolated populations experience declines or extirpation (Mammola et al. 2022). Here I present life history information for *F. antroecetes* gained through captive breeding under simulated cave conditions, the first such published account for a stygobitic snail.

Methods

Six individuals of *F. antroecetes*, ranging in size from 2 mm to 3 mm shell length, were collected from Stemler Cave, St. Clair County, Illinois USA between 28 August 2016 and 30 May 2017. The snails were variously paired in three different combinations in an attempt to induce reproduction in the laboratory under simulated cave conditions. *Fontigens antroecetes* is gonochoristic and females store sperm once mated. Because externally visible sexual dimorphisms are absent in this species, the sex of individual snails was not determined and it was not known whether any individuals had mated prior to collection.

Cave-like conditions were created within 15 × 10 × 5 cm lidded plastic containers. Each container included cave water and a cobble collected from the stream in Stemler Cave. Cobbles were inspected carefully to exclude any new snails or snail embryos. The cobbles included biofilms on which the snails grazed and substrates for egg deposition. The average cobble size was 12 × 9 × 3 cm. Water depth in the containers was maintained at 3.5 cm. The containers were aerated by aquarium air pumps with a tube fitted through a hole drilled in the lid. The containers were placed in a darkened 46 × 46 × 51 cm electronic cooler set at 13 °C, the mean annual water temperature in Stemler Cave (Taylor et al. 2000). The cooler was fitted with two shelves and could house a total of eight containers, four per shelf.

The experimental containers were removed from the cooler once or twice a week for data collection. The condition of the adult snails was assessed and all surfaces of the cobbles were inspected under a stereozoom microscope for the presence of eggs and to check the condition of developing embryos. The position of any newly deposited eggs was noted (top, side, or bottom surface of the cobble). Water was changed on average every 7.84 days (± 3.3 SD) and cobbles were replaced when biofilms were depleted and to isolate newly detected eggs. Cobbles were replaced on average every 10.26 days (± 6.13 SD). 38.46% of cobble changes were done to isolate newly deposited eggs. Embryonic development times were estimated because embryos were not checked daily to avoid excess handling and potential damage. Mean estimated development times were calculated by averaging the longest and shortest possible intervals between oviposition and hatching, which varied depending on the frequency of observation for each embryo. Newly hatched snails were isolated in separate containers as space in the cooler permitted. Growth rates of a limited number of offspring were estimated by periodically measuring shell length to nearest 0.25 mm with a ruler.

Similar procedures were followed using nine *F. antroecetes* collected from Cliff Cave in St. Louis County, Missouri (USA) on 22 December 2018. The snails were divided

equally between three containers. Water and cobbles from Stemler Cave were added and the containers were placed in simulated cave conditions on 8 January 2019. The null hypothesis that numbers of eggs laid on top, bottom and side of cobbles did not differ between Stemler Cave snails and Cliff Cave snails was evaluated using Fisher's exact test, followed by *post hoc* pairwise Fisher's exact test with Benjamini-Hochberg FDR method for correcting *p* values in R version 4.1.2 using packages "stats" and "statix" (R Core Team 2001). No regular growth rate data were collected from Cliff Cave offspring.

Results

Stemler cave snails

Wild collected snails from Stemler Cave produced a total of 49 eggs under simulated cave conditions. Eggs were deposited singly, within a clear capsule approximately 1 mm in diameter (Fig. 2). Eggs were invariably deposited within pits, crevices, or depressions on the surface of cobbles. The vast majority of oviposition (93.9%) occurred on the underside of cobbles. Mating and egg laying behaviors were not directly observed during this study.

Hatching success rate was 81.6%. A total of five embryos were accidentally damaged or destroyed during handling for data collection and four embryos failed to fully develop for unknown reasons. Mean estimated embryonic development time was 70.7 days (range 56.5 to 81 days). The uncertainty in development time ranged from 2 to 8 days, with an average of 5.3 days. The hatching process was directly observed in one instance. The snail became more active within the egg capsule in the two days preceding hatching and was observed chewing on the capsule. At the time of hatching the snail crawled out of the capsule through a well-formed hole that it had apparently created. This hatching process has also been documented by Davis (1961) in embryos of the North American freshwater hydrobioid *Amnicola limosa*, Say 1817. Newly hatched *F. antroecetes* individuals had a shell length of approximately 0.75 mm (Fig. 2). Survival of hatchlings was poor, and limited cooler space restricted the opportunity to collect growth rate data on isolated individuals. Data on shell length changes over time from several hatchling snails were combined to produce a growth profile over one year (Fig. 3). These data suggest that captive reared Enigmatic Cavesnails can reach the reported adult shell length of 2.5 mm (Hershler et al. 1990) in approximately 32 weeks.

Timelines and details about egg deposition for each of the three Stemler Cave snail pairings are presented below.

Pair 1 – Two snails collected from Stemler Cave on 28 August 2016 were housed in cave water at ambient room temperature (circa 20 °C) for 142 days, during which 10 eggs were deposited. The snails were transferred to simulated cave conditions on 17 January 2017. Pair 1 snails produced a single egg under simulated cave conditions on 21 February 2017, which failed to develop to hatching. The pair was maintained for

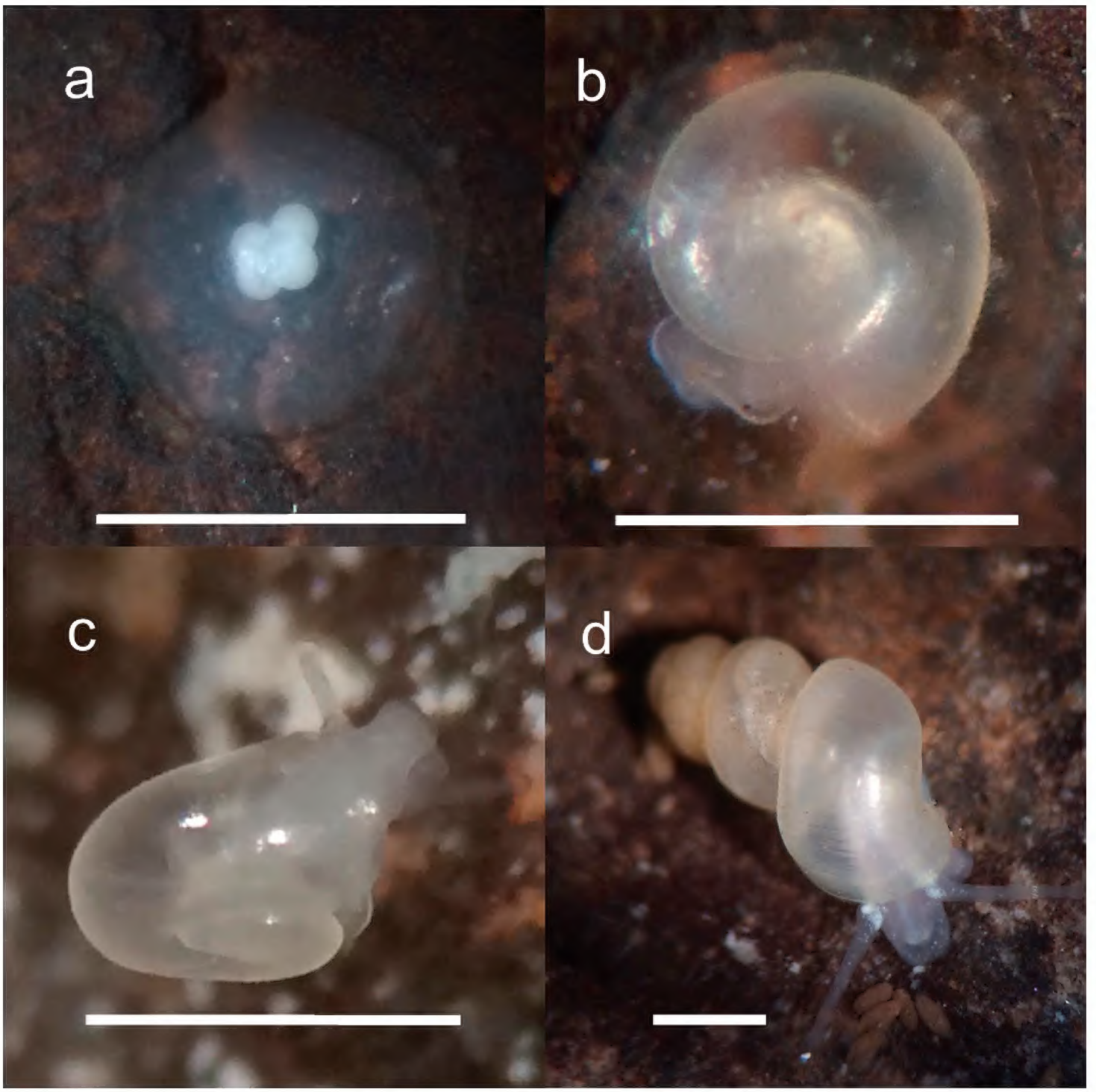

Figure 2. Life stages of *Fontigens antroecetes* observed under simulated cave conditions in the laboratory **a** egg capsule containing two-day old embryo at the four-celled stage **b** well developed embryo at 75 days development **c** newly hatched snail **d** adult lab raised individual. Scale bars represents approximately 1 mm.

total of 70 days until one of the adults was accidentally crushed during data collection on 4 April 2017. The remaining snail was maintained alone under the simulated cave conditions until it was paired with another specimen on 30 May 2017.

Pair 2 – Three snails were collected from Stemler Cave on 4 March 2017 and immediately combined under simulated cave conditions. One snail was found dead on 28 March 2017. The remaining two snails were maintained for 181 days and produced 40 embryos. Egg production appeared to be cyclical during the 25 weeks that the snails were maintained (Fig. 4). Four embryos were damaged due to mishandling and three failed to fully develop. On 1 September 2017 one of the snails was found dead and the remaining individual was then preserved.

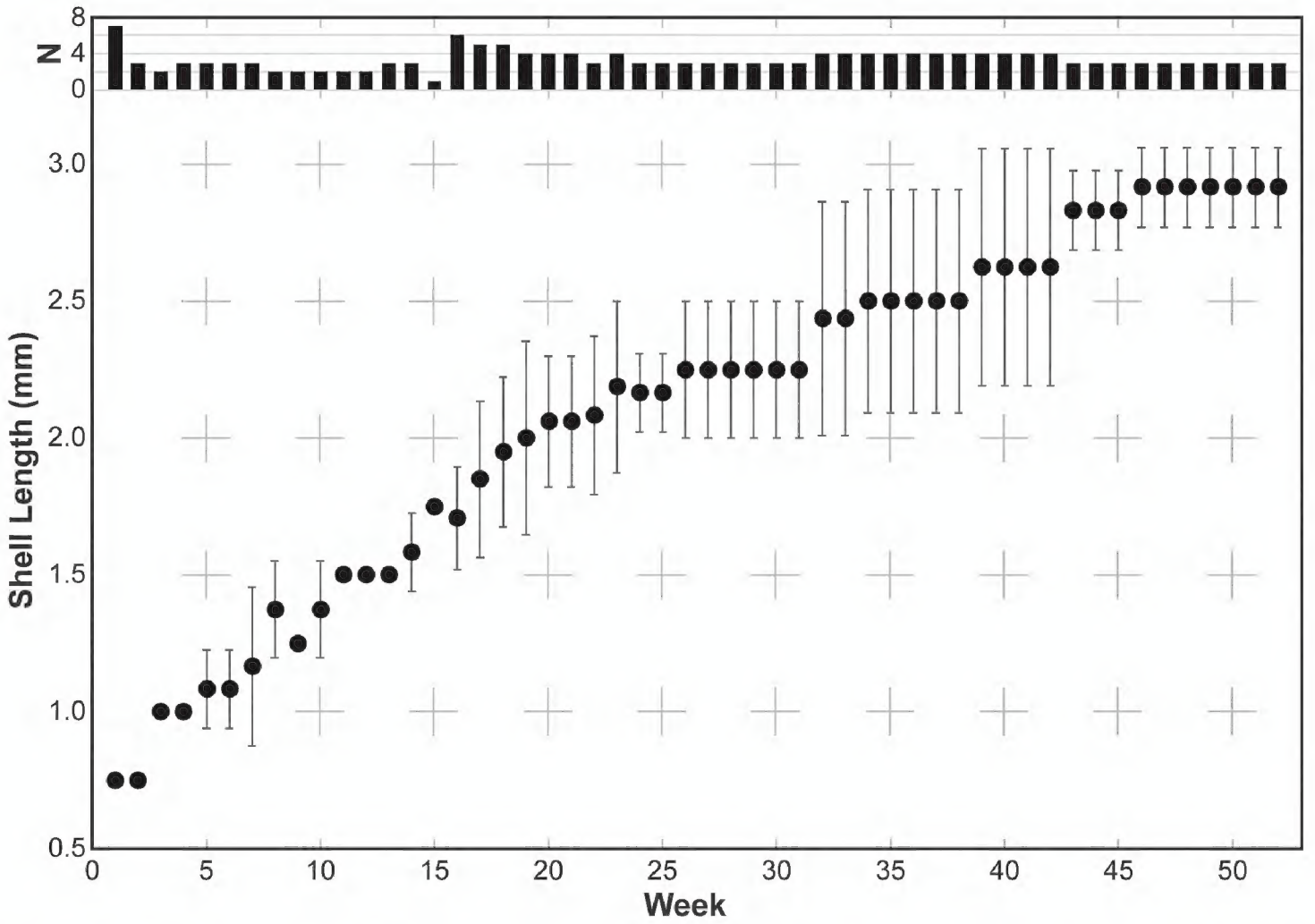

Figure 3. Growth profile of *Fontigens antroecetes* held under simulated cave conditions for up to 52 weeks post hatching. Error bars represent +/- one standard deviation. Histogram indicates number of individuals measured at each week interval.

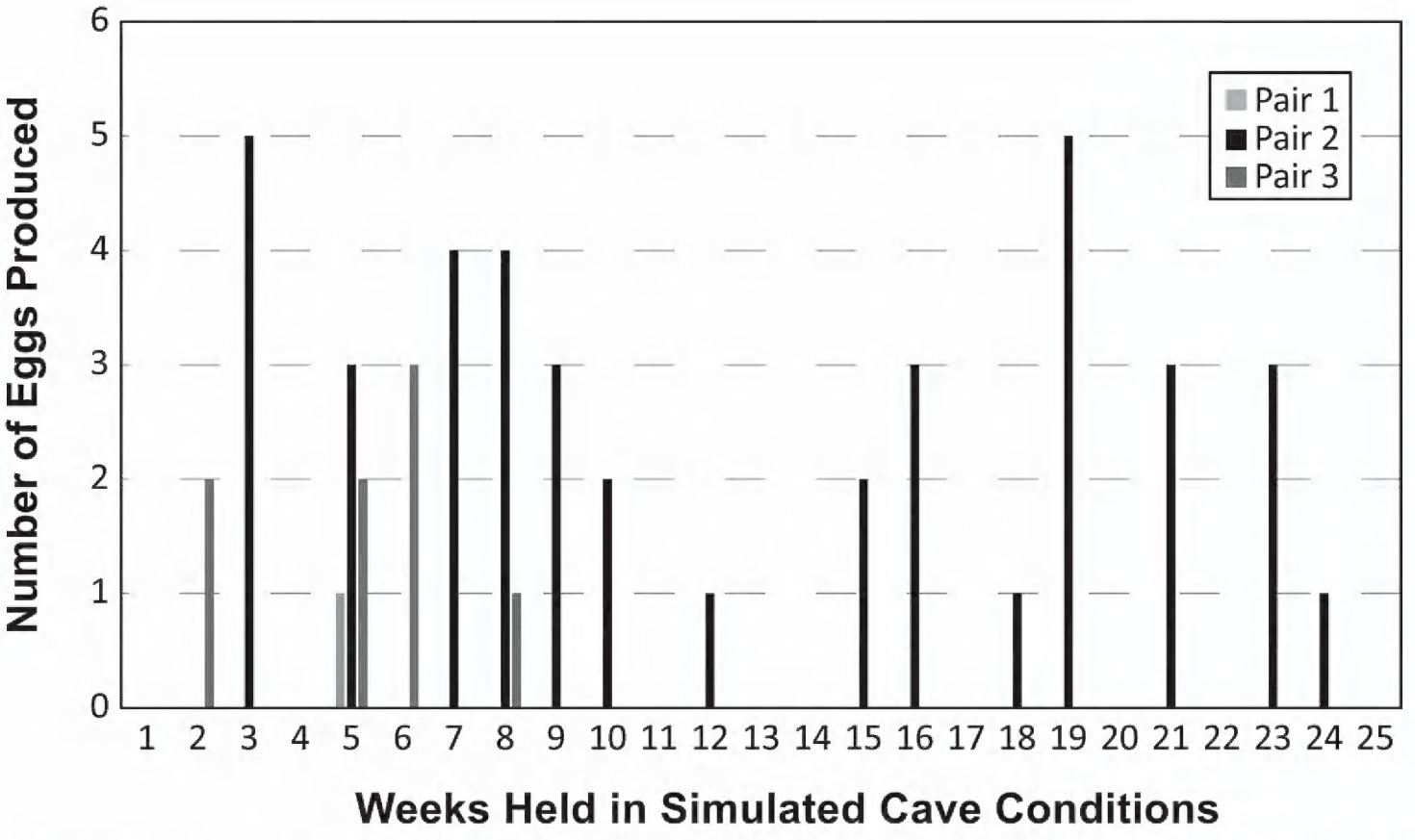

Figure 4. Pattern of egg production by *Fontigens antroecetes* from Stemler Cave, Illinois over 25 weeks in simulated cave conditions.

Pair 3 – A single snail was collected from Stemler Cave on 30 May 2017 and combined with the surviving individual from Pair 1. The pair produced eight embryos during 61 days in simulated cave conditions. All embryos successfully developed to hatching. Both snails were found dead on 31 July 2017.

On 27 June 2019 two laboratory-produced offspring snails, raised in isolation for over two years, were combined in an attempt to yield a second generation of lab-reared offspring. The first egg produced by this pairing was deposited on 10 July 2019, at which time the parents were isolated in an attempt to determine the sex of each snail. A total of 13 eggs were deposited by the female between 10 July and 30 September 2019. Nine embryos successfully hatched (70%). Like the wild collected snails, the female deposited single eggs within in a gelatinous capsule, almost exclusively on the underside of cobbles, often in small pits or crevices. Only one egg was deposited on the top surface of a cobble. The mean estimated development time for the second generation was 73 days. The female, progeny of Pair 1, hatched on 11 December 2016, was 2.5 years old at the time of pairing, and had a shell length of 3 mm. The male, progeny of Pair 2, hatched circa 30 September 2017, was 2.25 years old, and had a shell length of 2.75 mm. The adult female survived until 21 October 2019 and likely died of injuries due to mishandling. The male survived until 22 April 2021. Two sibling second generation lab-reared snails were then reared together. These snails hatched in November 2019 and reached 2.5 mm shell length by the end of November 2020. This pair produced the first viable embryo in September 2021.

Some information about *F. antroecetes* reproduction in Stemler Cave can be gleaned from observations of embryos already attached to rocks collected from the cave stream for potential use in the captive breeding efforts. Well-developed embryos, apparently near hatching, were observed on 14 October 2016, 11 December 2016, 21 June 2017, and 13 November 2021. Early stage embryos were seen on 13 November 2016, 18 December 2017, 29 December 2020, and 13 November 2021. Partly developed embryos were observed on 13 February 2018 and 13 November 2021.

Cliff cave snails

Captive Cliff Cave snails deposited a total of 34 eggs during the course of this study. The first egg was observed on 14 February 2019 and the last on 6 March 2020. All eggs were deposited singly, within an approximately 1 mm wide gelatinous capsule. Unlike the pattern seen in Stemler Cave snails, the Cliff Cave animals mostly utilized pits and crevices on the top and side surfaces of cobbles for oviposition (Fig. 5). Embryonic development time was on average longer (mean estimated time of 82.17 days) and more variable (range 63.5 to 119 days) in the Cliff Cave snails. The uncertainty in development time for Cliff Cave snail embryos ranged from a 2.5 to 14 days day, with an average of 6.5 days. Hatching success rate was 90%. Hatchling shell length was approximately 0.75 mm.

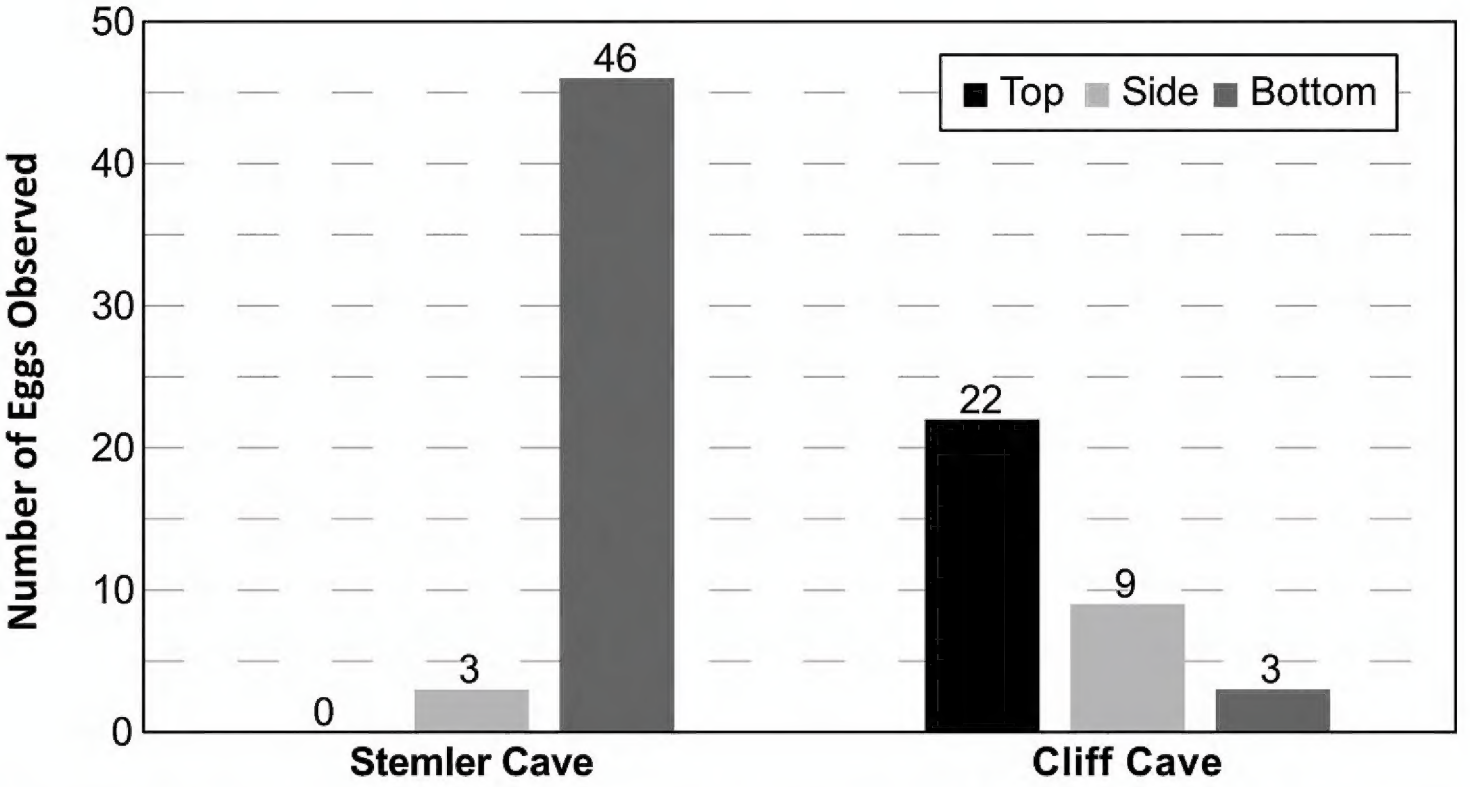

Figure 5. Comparison of egg deposition on top, side, and bottom surface of cobbles in captive *Fontigens antroecetes* from Stemler Cave, IL and Cliff Cave, MO under simulated cave conditions.

Timelines and details about egg deposition for each of the three Cliff Cave snail combinations are presented below.

Container 1 – The three snails in container 1 produced no eggs after 225 days in captivity when all were preserved on 4 August 2019.

Container 2 – A total of six eggs were produced with the first egg observed 14 February 2019, 37 days after the snails were placed in simulated cave conditions. One snail was found dead on 2 May 2019. One additional egg was deposited beyond that date. A second snail was accidentally crushed during handling on 27 June 2019. No additional eggs were deposited beyond that date, and the remaining snail was preserved on 4 August 2019.

Container 3 – The first egg was discovered on 18 April 2019, 100 days after the snails were placed in simulated cave conditions. Sixteen additional eggs were deposited between 18 April and 16 July 2019. At that time, each of the three snails were sequentially isolated in an attempt to gain information about egg production by individuals. The first snail isolated produced no eggs before it was preserved on 6 August 2019. The two remaining snails were left paired between 16 July and 30 July 2019, during which four additional eggs were produced. These two individuals remain alive at this time, over 1000 days after entering simulated cave conditions. One produced a single additional egg on 18 August 2019. The remaining snail produced six additional eggs, with the last egg deposited on 6 March 2020.

A few Cliff Cave offspring were successfully raised to maturity. Three individuals hatched in September 2019 had reached 2.75 mm shell length by July 2020 and began

producing a second generation in the lab by late August/early September 2020. Twenty-six eggs were observed between September 2020 and September 2021. Oviposition varied from the wild-collected snails in that a higher percentage of eggs were deposited on the bottom side of cobbles (54%). One surviving individual continued to produce viable embryos in September 2021.

Discussion

Egg production and oviposition

The deposition of single eggs within capsules, attached to substrates appears to be typical for several genera of hydrobioid snails (Johnson et al. 2013). The two by three matrix of oviposition locations on cobbles differed between Stemler Cave and Cliff Cave snails ($p < 0.0000$), with *post hoc* pairwise Fisher's exact tests demonstrating marked differences between caves for both bottom versus side (n = 61, adjusted $p < 0.0000$) and bottom versus top (n = 71, adjusted $p < 0.0000$) and less pronounced differences between side and top (n = 34, adjusted $p = 0.0368$) of cobbles. The differences in oviposition behavior observed between the Stemler Cave and Cliff Cave snails in this study could be explained by differences in the physical conditions in the two caves. Unlike Stemler Cave, the gravel and cobble substrates in Cliff Cave are largely cemented together with calcium carbonate deposits, limiting interstitial spaces and making the undersurfaces of cobbles less accessible to snails. The tendency for Cliff Cave snails to deposit eggs on the upper and side surfaces of cobbles could be a response to the high degree of calcium deposition covering the streambed, limiting access to the undersurface of substrates. Stemler Cave has a large recharge basin (18 Km^2) and is subject to intense pulse floods following heavy rains. Oviposition on the protected undersides of substrates could be an adaptation in Stemler Cave snails to intense stream flow.

Almost nothing is known about stygobitic snail reproduction in nature. Slay et al. (2016) reported that *Fontigens* in the Ozarks have been observed depositing eggs in April. My observations of embryos attached to rocks collected from Stemler Cave suggest that reproduction in that population may be on ongoing and year-round. This is in accordance with observations by Bichuette and Trajano (2003), demonstrating that two species of subterranean hydrobioids in the genus *Potamolithus* showed non-seasonal reproduction with a high percentage of mature females present throughout the year.

Growth rates and longevity

Mean estimated embryonic development time of 70.7 days for Stemler snails and 82.17 days for Cliff Cave snails is surprisingly long considering the small size of the embryo, and likely reflect slow growth and lower metabolic rates typical of cave-adapted organisms (Culver and Pipan 2009). I have conducted unpublished studies of reproduction and growth of *Physella acuta* (Draparnaud, 1805) from Stemler Cave in the laboratory

under simulated cave conditions. *Physella acuta* is a snail known for its ability to invade new habitats (Dillon et al. 2006) and only opportunistically inhabits caves. *Physella acuta* had a considerably faster life cycle under simulated cave conditions compared to *F. antroecetes*. Mean embryonic development time was 18.5 days, and snails grew to adult size and began reproducing after approximately 60 days. Weck and Taylor (2016) reported similar patterns with another cave-dwelling population of *Physella* (likely *P. gyrina*) from southwestern Illinois.

No comparable studies of sygtobitic snail growth and longevity are available, although a few studies of epigean hydrobioids have been published. *Potamopyrgus antipodarum* (Gray 1843) reared in aquaria at 12 °C grew at a rate of 0.007 mm/day over 250 days, and had a predicted life span of 16–18 months based on observations of a wild population (Dahl and Winter 1993). In this study, the average growth rate of *Fontigens antroecetes* raised at 13 °C was identical (0.007 mm) over the same period of time (250 days). Lassen and Clark (1979) raised three species of marine hydrobioids in the laboratory. Development time for *Hydrobia* spp. was 12–15 days at 15 °C, the field temperature during peak reproduction in nature. Lab-produced *Ecrobia ventrosa* (Montagu, 1803) offspring reared at 15 °C began producing second generation eggs after 13 weeks. Development times more comparable to those observed in my study (49 to 70 days) were only seen when temperatures were reduced to 4 °C. Some parallels to the trends observed in *F. antroecetes* are also seen the amnicolid hydrobioids living in the cold (3.6 °C) temperatures of Lake Baikal. Ropstorf and Sitnikova (2006) made *in situ* observations that showed year-round reproduction, long development times (several months) and slow growth rates, with up to 4 years to maturity for Baikalian snails.

Brown et al. (2008) considered North American freshwater hydrobioids to be annuals with seasonal recruitment in cold systems. *Amincola limosa* Say 1817 and *Pomatiopsis cincinnatiensis* (Lea 1840) both exhibit seasonal reproduction and an annual life cycle (Van der Schalie and Getz 1962; Pinel-Alloul and Magnin 1973). The observations in this study suggest that *F. antroecetes* is not an annual, and follows life history strategies typical of other cave-adapted animals: slow growth, late age at maturation, and long lifespan (Fiser 2019). Enigmatic cavesnails take approximately 10 weeks to complete embryonic development, 32 weeks to reach minimum reported adult size, at least one year to reach sexual maturity, and can survive for at least 3 years in captivity. A life cycle of 2–3 years for this species is likely.

The results obtained in this study provide a possible approach to gaining life history information about snails from other subterranean systems. Dr Paul Johnson (personal communication, 2 December 2021) attempted to develop protocols for captive rearing the federally endangered Tumbling Creek Cavesnail, *Antrobia culveri*, Hubricht, 1971, at the Tennessee Aquarium. The project utilized *Antrorbis breweri*, Hershler & Thompson, 1990, the Manitou Cavesnail from Alabama, and *Fontigens* spp. as surrogates. They were able to propagate some snails, but not in appreciable numbers sufficient for repopulating a portion of a cave. Future studies could explore more effective methods for maintaining laboratory populations of stygobitic snails as a possible refugium to protect against catastrophic events such as chemical spills. These

laboratory populations could then provide a source for reintroduction after the cave stream has sufficiently recovered. This approach has been used for subterranean aquatic species in the Edwards Aquifer of Texas (Britton et al. 2020). These future studies of laboratory culture of *F. antroecetes* should explore refinement of methods for handling of cobbles and specimens to reduce accidental mortalities from handling and improve recruitment of hatchling snails.

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